Cell, Vol. 108, 5–8, January 11, 2002, Copyright ©2002 by Cell Press

Muscular Dystrophy—Reason for Optimism?

Minireview

Edward A. Burton[1] and Kay E. Davies[2,3,4]
[1] Department of Clinical Neurology
[2] Department of Human Anatomy and Genetics
[3] MRC Functional Genetics Unit
University of Oxford
Oxford OX1 3QX
United Kingdom

Characterization of the mechanisms underlying various types of muscular dystrophy has been an outstanding triumph of molecular biology. Increasing clarification of the aberrant cellular processes responsible for these conditions may ultimately permit the development of effective means for molecular intervention, allowing correction of the abnormal cellular physiology that results in the dystrophic phenotype.

Muscular dystrophies are a heterogeneous group of inherited single-gene disorders, characterized clinically by progressive muscle weakness and wasting. Until recently, this large group of conditions was classified according to Mendelian inheritance patterns and clinical features. With the advent of molecular genetic mapping techniques, however, it became clear that a number of clinically similar conditions were linked to an array of distinct genomic loci. Recent advances in positional cloning, bioinformatics, and candidate gene analysis have started to elucidate the mechanisms responsible for various types of muscular dystrophy and have shed some light onto the (often related) functions of the many different gene products implicated in the etiology of these diseases. Elucidation of molecular pathways involved in the pathogenesis of muscular dystrophy has enabled the rational conception of strategies aimed at alleviating the dystrophic phenotype. Here, we examine what is known about the molecular pathogenesis of the muscular dystrophies and review strategies aimed at averting the muscle degeneration that results from an assorted group of primary genetic defects.

Dystrophin

The first breakthrough in understanding the molecular basis for muscular dystrophy happened in 1986. Following a long, multinational effort, the gene defect responsible for the common X-linked severe childhood-onset Duchenne type of muscular dystrophy (DMD) was established by positional cloning techniques, in Kunkel's laboratory. The gene product, dystrophin, is a large cytoskeletal protein that is located at the cytoplasmic face of the muscle cell plasma membrane. Dystrophin is missing from the muscles of DMD patients. The milder allelic disorder, Becker muscular dystrophy (BMD), is caused by mutations that result in reduced expression of dystrophin, or expression of truncated partially functional forms of the protein. Analysis of the primary sequence of dystrophin predicted an N-terminal domain sharing homology with α-actinin, and a large central region containing spectrin-like repeats, predicted to give the molecule a rigid, rod-shaped structure. The N-terminal domain was subsequently shown to bind the F actin component of the muscle cytoskeleton. The C-terminal domain did not show homology with previously described proteins, and its function remained enigmatic (for reviews of dystrophin structure and function with primary references, see Burton and Davies, 2000; Blake et al., 2001).

Dystrophin-Associated Proteins

The next important piece in the puzzle fell into place when the laboratories of Campbell and Ozawa demonstrated that the C-terminal part of the dystrophin molecule bound to a complex of novel glycoproteins embedded in the muscle cell membrane. Subsequent work from both of these laboratories, and others, has shown that the dystrophin-associated glycoprotein complex (DPC) contains three subcomplexes of proteins. The complex is depicted schematically in Figure 1 (for reviews of the DPC with primary references, see Blake et al., 2001; Burton and Davies, 2000): α- and β-dystroglycan are derived from a single protein precursor by post-translational processing. β-dystroglycan is a transmembrane protein; the intracellular C-terminal domain binds directly to dystrophin. The extracellular domain of β-dystroglycan binds to α-dystroglycan. The latter is heavily glycosylated, wholly extracellular, and binds to the basement membrane component laminin. This completes a molecular link between the myofiber F actin cytoskeleton and the extracellular matrix (ECM), via dystrophin and α- and β-dystroglycan.

α-, β-, γ-, δ-, ϵ-sarcoglycans and sarcospan are integral membrane glycoproteins forming a subcomplex that associates with β-dystroglycan. The functions of the sarcoglycan complex are uncertain. The close association between sarcoglycans and other members of the DPC suggested that mutations in the genes encoding these glycoproteins might be responsible for other forms of muscular dystrophy. Loss-of-function mutations in genes encoding the sarcoglycans have been shown to cause various types of recessive limb girdle muscular dystrophy (LGMD) and severe childhood onset muscular dystrophy (reviewed in Bushby, 1999). In many cases, loss of one component seems to destabilize the entire DPC and lead to disruption of the cytoskeleton–extracellular-matrix link, implying that one function of SGs might be in maintaining stability of the DPC.

α- and β- syntrophin and α-dystrobrevin are intracellular proteins that associate with the C terminus of dystrophin. Syntrophin seems to recruit signaling proteins to the DPC, including nNOS and voltage-gated sodium channels. Novel binding partners of dystrobrevin are discussed below. No pathogenic mutations have been described in the genes encoding syntrophin or dystrobrevin.

Functions of Dystrophin

The cytoskeleton-dystrophin-dystroglycan-ECM link has been postulated to perform a mechanical function in muscle, providing protection for the sarcolemma against the local forces that develop during muscle con-

[4] Correspondence: kay.davies@anat.ox.ac.uk

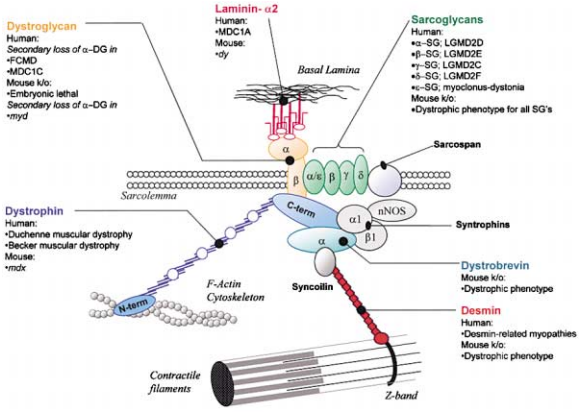

Figure 1. Schematic Depiction of the Dystrophin-Associated Complex

traction. In support of this idea, the absence of dystrophin renders the sarcolemma susceptible to contraction-induced rupture. One prediction from this is that defects of other components of the cytoskeleton-ECM link would result in muscle degeneration. This is, in fact, the case; genetic mutations abolishing production of the basement membrane protein laminin give rise to a congenital form of muscular dystrophy in which the phenotype extends to peripheral nerve and central nervous system pathology. Interestingly, no naturally occurring mutations have yet been described in the dystroglycan gene. Mouse dystroglycan null mutations are lethal in early embryogenesis, owing to a failure to form an essential extra-embryonic structure, Reichert's membrane (Williamson et al., 1997). Muscle-specific loss of dystroglycan, and antibody-mediated disruption of the link between muscle α-dystroglycan and laminin, however, both cause muscular degeneration in mouse models, implying that the normal function of DG is essential to maintain myofiber integrity (reviewed in Burton and Davies, 2000).

Dystrophin almost certainly has functions other than maintaining the cytoskeleton-ECM link. Secondary loss of the DPC occurs in dystrophin-deficient muscle. In other circumstances, however, it is possible to dissociate loss of the DPC and loss of dystrophin. Transgenic mice, which are null for full-length dystrophin but express the C-terminal domain in muscle, maintain the DPC at the sarcolemma, but develop muscular dystrophy. This is consistent with the cytoskeletal-ECM link performing a crucial function. However, loss of DPC components in the presence of a normal cytoskeleton-ECM link can also give rise to muscular dystrophy. For example, although no mutations have been described in the human α-dystrobrevin (DB) gene, α-DB null mice develop dystrophic muscle pathology; the cytoskeleton-ECM link remains uninterrupted (Grady et al., 1999). This might be attributable to the absence of the loss of crucial signaling molecules from the α-DB null complex, but recent data implies that loss of novel cytoskeletal links may be an important factor. Syncoilin is a newly identified component of the DPC that interacts with both α-DB (Newey et al., 2001) and the muscle intermediate filament protein desmin (Poon et al., 2001). The latter is

Table 1. Muscular Dystrophy Can Result from Mutations in Genes Whose Products Are Not Known to Associate with Dystrophin or the DPC

Gene product	Location	Phenotypes
Emerin	Nuclear envelope	X-EDMD
Lamin A/C	Nuclear envelope	AD-EDMD
		LGMD 1B
Dysferlin	Membrane	Distal myopathy
		LGMD 2B
Caveolin 3	Caveolae	LGMD 1C
Calpain 3	Cytoplasm	LGMD 2A
Integrin α7	Membrane	Congenital myopathy
Collagen VI	ECM	Bethlem myopathy
DMPK	Neuromuscular junction	Myotonic dystrophy
ZNF9	Nuclear?	PROMM
PABP2	Nuclear	OPMD
Telethonin	Sarcomeric	LGMD 2G
Myotilin	Sarcomeric	LGMD 1A

Abbreviations: EDMD, Emery-Dreifuss muscular dystrophy; LGMD, limb girdle muscular dystrophy; DMPK, myotonic dystrophy protein kinase; PROMM, proximal myotonic myopathy; OPMD, oculopharyngeal muscular dystrophy; and PABP2, polyA-binding protein 2.

part of an intermediate filament network, which links the muscle contractile apparatus to the sarcolemma. The properties and functions of the α-DB–syncoilin–desmin link are unclear at present, but further evaluation may reveal more interesting functions of the DPC. Observations such as these imply that the consequences of dystrophin deficiency are complex and may be caused both by direct loss of its cytoskeletal functions and by secondary loss of DPC components. There is some evidence that dystrophic muscle fibers have abnormal trans-sarcolemmal calcium currents, and higher intracellular calcium concentrations than normal fibers, but there is no universal agreement about the presence or nature of such changes, or whether they are primary or secondary to muscle damage from other factors (reviewed in Burton and Davies, 2000).

Muscular Dystrophy Not Linked to Loss of Dystrophin or Associated Proteins

Although many types of muscular dystrophy have been linked with loss of function of dystrophin and its associated proteins, this is by no means an exclusive mechanism for the production of hereditary muscle degeneration. A series of disparate cellular pathways, whose disruption is associated with various forms of muscular dystrophy, has been identified. Examples are summarized in Table 1. Interestingly, links have emerged between several members of this group. For example, emerin and lamin A/C are both nuclear envelope proteins (reviewed in Nagano and Arahata, 2000), and there is evidence that the membrane-associated proteins dysferlin and caveolin-3 interact.

Many of the implicated molecules have no obvious link with the DPC at present; some may represent components of independent pathways that result in a shared pathological phenotype. Further study will elucidate how far downstream from the primary genetic defect any biochemical convergence occurs.

Posttranscriptional Mechanisms and Muscular Dystrophy

An interesting story has emerged from study of the most common type of hereditary myopathy, myotonic dystro-

phy. This autosomal dominant condition is caused by an expanded trinucleotide repeat sequence in the 3′UTR of the DMPK gene, which encodes a serine/threonine kinase. The mutant mRNA is retained within myonuclei, where it aggregates in discrete foci and may interfere with export of the wild-type mRNA. However, reduced levels of DMPK protein do not explain the phenotypic changes fully; murine DMPK null mutants do not develop muscular dystrophy, myotonia, or cataract (although they do develop cardiac conduction defects). Interestingly, the expanded triplet repeat causes changes in chromatin structure, reducing expression of a transcription factor (*six5*) encoded at an adjacent genomic locus. Murine six5 null mutants develop cataract, potentially explaining part of the pleiotropic DM phenotype. Recent work suggests that muscle wasting and myotonia may be attributable to a toxic gain of function of the DMPK RNA, because of the expanded repeat sequence. The DM phenotype is reproduced in a mouse model, in which the expanded CUG repeat is placed within a transgene encoding human skeletal actin, which is unrelated to DMPK. Toxic gain of function at the RNA level may be a general mechanism resulting in this phenotype. A similar dominant disease to DM (proximal myotonic myopathy) has been described in patients with an expanded CCUG tetra nucleotide repeat within the first intron of the gene encoding a zinc finger transcription factor, ZNF9. This gene is unrelated to DMPK and is not located near the *six5* gene, implying that gain of RNA function is the pathogenic mechanism (reviewed in Tapscott and Thornton, 2001).

Posttranslational Processing and Muscular Dystrophy

Posttranslational modifications of muscle cell proteins appear to be just as important as the presence of the correct primary amino acid sequences in ensuring that essential components of muscle are assembled in an orderly fashion and function correctly.

Muscle-eye-brain disease (MEB) is a type of congenital muscular dystrophy associated with loss-of-function mutations in the gene encoding a glycosyltransferase, POMGnT1 (Yoshida et al., 2001). This enzyme is responsible for the addition of N-acetlyglucosamine moieties to O-mannosyl glycoproteins. It is known that sialylated O-glycosidically-linked oligosaccharides of α-dystroglycan are involved in its interaction with the basement membrane component laminin, and it has thus been speculated that POMGnT1 is the enzyme responsible for this important protein modification. Furthermore, the congenital muscular dystrophy syndrome, Fukuyama congenital muscular dystrophy (FCMD), is associated with specific secondary loss of heavily glycosylated α-dystroglycan (Hayashi et al., 2001). The product of the FCMD gene, fukutin (Kobayashi et al., 1998), has an unknown function. Fukutin has a homolog in muscle, fukutin-related protein (FKRP), which is affected by loss-of-function mutations in another form of congenital muscular dystrophy (Brockington et al., 2001), and in a milder allelic disorder, limb girdle dystrophy type 2I. Both fukutin and FKRP contain conserved motifs present in glycosyltransferases, and it remains possible that they are members of this enzyme family.

A further, recently characterized example of a disease-producing mutation in a gene whose product is involved in protein modification is provided by hereditary inclusion body myopathy. This is an unusual type of late-onset muscle disease, which is prevalent in Jewish populations and is caused by mutations in gene encoding UDP-N-acetylglucosamine-2-epimerase/N-acetylmannosamine kinase, the rate-limiting enzyme responsible for sialic acid synthesis (Eisenberg et al., 2001). Sialic acid residues are a common addition to glycoproteins, and are present within O-linked sugar moieties of α-dystroglycan. The absence of the apparatus for their synthesis would preclude their addition to nascent proteins. Finally, the mouse muscular dystrophy mutant, *myd*, may well share a similar pathogenic mechanism. The muscle wasting phenotype in this animal model is caused by loss-of-function mutations in LARGE (Grewal et al., 2001). The predicted gene product shows homology to various types of eukaryotic and prokaryotic glycosyltransferase, and there is secondary loss of α-dystroglycan glycosylation in the muscles of *myd* mice.

New Therapeutic Approaches

Following elucidation of the molecular basis for DMD, and some types of LGMD, it was hoped that gene therapy would enable restoration of a normal muscle phenotype by replacing the aberrant cellular copy of the relevant gene. It has become apparent that delivery of a therapeutic transgene to all of the muscles in the body is a tall order. In addition, there are important immunological consequences from expressing a protein in an individual who does not possess a functional copy of the relevant gene, in that the resulting immune response to the "foreign" protein tends to curtail transgene expression. Further problems include the immune response to vector components and the special problems for DMD gene therapy, introduced by the size of the dystrophin cDNA (14 kb). Consequently, a series of creative strategies has been developed to alleviate the progressive muscle weakness and wasting that characterizes muscular dystrophy. Many of these approaches have been developed with specific types of muscular dystrophy in mind, whereas others may have general applicability.

The function of a missing protein may be undertaken by a different protein. Two broad strategies have been conceived to take advantage of this possibility for therapeutic purposes. First, instead of directly replacing the missing gene product, it may be possible to generate artificial constructs that alleviate muscle pathology by performing a similar function to the absent protein. A simple example is provided by the use of truncated dystrophin minigenes for virally mediated delivery in DMD. A more sophisticated approach has been described by Ruegg and colleagues, who studied a model of congenital muscular dystrophy, the laminin α2 deficient dy^w mouse (Moll et al., 2001). Muscle degeneration in this animal results from failure of the interaction between dystroglycan and the ECM, owing to loss of the important ECM ligand for α-dystroglycan. A minigene based on another ECM component, agrin, was designed to bind to both (1) other components of the basement membrane and (2) dystroglycan, thus restoring the DG-ECM link. Transgenic overexpression of the minigene in the muscles of dy^w mice prevented the muscle pathology, indicating that a rationally designed, engineered gene product could replace the function of the missing

protein. Second, protein replacement might be achieved by increasing the expression of an endogenous homolog. Dystrophin has an autosomal paralog, utrophin. The latter is expressed at low levels in adult muscle, but at much higher levels in fetal muscle, where it is localized to the sarcolemma. The two proteins are functionally redundant, as shown in transgenic mouse experiments in which dystrophin null mice are spared the expected muscle degeneration by overexpression of a utrophin transgene in muscle (Tinsley et al., 1998). A detailed characterization of the processes regulating utrophin expression in muscle is currently underway, with the aim of identifying pharmacological means to enforce its upregulation in DMD patients' muscles.

Much of the late muscle pathology in DMD is related to a failure of ongoing muscle regeneration, with a consequent loss of muscle fiber bulk. Targeting this process might be advantageous in that benefits gained through sustained myoregenerative capacity would not necessarily be specific to a particular disease. Rosenthal and colleagues have recently shown that transgenic overexpression of a tissue-restricted form of insulin-like growth factor 1, expressed in skeletal muscle, is able to enforce sustained hypertrophy and regeneration in senescent muscle (Musaro et al., 2001). This finding raises the exciting prospect of alleviating the functional deficit in muscle by sustaining its capacity to renew myofibers that are lost during the dystrophic process. Conceivably, this might be achieved through pharmacological upregulation of the IGF-1 muscle isoform, circumventing the necessity to deliver transgenes to muscle.

Finally, a detailed understanding of the molecular pathways involved in the pathogenesis of each of the muscular dystrophies might enable identification of specific processes that would be amenable to biochemical intervention using drugs. This approach would not seek to correct the underlying molecular deficit, but to control its consequences. Work in this area has commenced with the recent publication of a microarray analysis designed to identify groups of genes whose expression may be dysregulated in muscular dystrophy (Chen et al., 2000). The result was a bewildering selection of seemingly unrelated genes that were either up- or downregulated in dystrophic tissue. The next task is to align these gene products into an orderly series of secondary expression cascades and to identify which are pathogenic. This work is exciting, as pharmacological manipulation of the secondary consequences of a genetic mutation might not require either gene delivery or enforced therapeutic changes in gene expression.

Conclusions

The past fifteen years have witnessed unprecedented advances in our understanding of the cellular processes giving rise to muscular dystrophy, but several challenges remain. Our knowledge of the molecular mechanisms responsible for some forms of muscular dystrophy remains incomplete, and elucidation of the underlying reasons for muscle degeneration in diseases like facio-scapulohumeral muscular dystrophy is a priority. In relation to the growing list of conditions for which there is an established underlying primary genetic defect, a better understanding of the secondary pathogenic cascades may allow the identification of molecular targets for the actions of drugs. Development of an effective way to deliver therapeutic transgenes to skeletal muscle would allow many of the diseases to be treated either directly or indirectly by gene therapy, and the development of pharmacological methods for manipulating the expression of endogenous genes might allow alleviation of muscle pathology through enforced upregulation of disease gene homologs, or downregulation of effector pathways.

In contrast to the situation two decades ago, we now at least have an idea of the mechanisms leading to muscular dystrophy, and the strategies by which we might tackle the resulting aberrant molecular processes. In this regard, there is reason for cautious optimism.

Selected Reading

Blake, D.J., Weir, A., Newey, S.E., and Davies, K.E. (2001). Physiol. Rev., in press.

Brockington, M., Blake, D.J., Prandini, P., Brown, S.C., Torelli, S., Benson, M.A., Ponting, C.P., Estournet, B., Romero, N.B., Mercuri, E., et al. (2001). Am. J. Hum. Genet. *69*, 1198–1209

Burton, E.A., and Davies, K.E. (2000). In The Pathogenesis of Neurodegenerative Disorders, M.P. Mattson, ed. (Totowa, NJ: Humana Press Inc.), pp. 239-284.

Bushby, K.M. (1999). Brain *122*, 1403–1420.

Chen, Y.W., Zhao, P., Borup, R., and Hoffman, E.P. (2000). J. Cell Biol. *151*, 1321–1336.

Eisenberg, I., Avidan, N., Potikha, T., Hochner, H., Chen, M., Olender, T., Barash, M., Shemesh, M., Sadeh, M., Grabov Nardini, G., et al. (2001). Nat. Genet. *29*, 83–87.

Grady, R.M., Grange, R.W., Lau, K.S., Maimone, M.M., Nichol, M.C., Stull, J.T., and Sanes, J.R. (1999). Nat. Cell Biol. *1*, 215–220.

Grewal, P.K., Holzfeind, P.J., Bittner, R.E., and Hewitt, J.E. (2001). Nat. Genet. *28*, 151–154.

Hayashi, Y.K., Ogawa, M., Tagawa, K., Noguchi, S., Ishihara, T., Nonaka, I., and Arahata, K. (2001). Neurology *57*, 115–121.

Kobayashi, K., Nakahori, Y., Miyake, M., Matsumura, K., Kondo Iida, E., Nomura, Y., Segawa, M., Yoshioka, M., Saito, K., Osawa, M., et al. (1998). Nature *394*, 388–392.

Moll, J., Barzaghi, P., Lin, S., Bezakova, G., Lochmuller, H., Engvall, E., Muller, U., and Ruegg, M.A. (2001). Nature *413*, 302–307.

Musaro, A., McCullagh, K., Paul, A., Houghton, L., Dobrowolny, G., Molinaro, M., Barton, E.R., Sweeney, H.L., and Rosenthal, N. (2001). Nat. Genet. *27*, 195–200.

Nagano, A., and Arahata, K. (2000). Curr. Opin. Neurol. *13*, 533–539.

Newey, S.E., Howman, E.V., Ponting, C.P., Benson, M.A., Nawrotzki, R., Loh, N.Y., Davies, K.E., and Blake, D.J. (2001). J. Biol. Chem. *276*, 6645–6655.

Poon, E.N., Howman, E.V., Newey, S.E., and Davies, K.E. (2001). J. Biol. Chem. *1*, 1.

Tapscott, S.J., and Thornton, C.A. (2001). Science *293*, 816–817.

Tinsley, J., Deconinck, N., Fisher, R., Kahn, D., Phelps, S., Gillis, J.M., and Davies, K. (1998). Nat. Med. *4*, 1441–1444.

Williamson, R.A., Henry, M.D., Daniels, K.J., Hrstka, R.F., Lee, J.C., Sunada, Y., Ibraghimov Beskrovnaya, O., and Campbell, K.P. (1997). Hum. Mol. Genet. *6*, 831–841.

Yoshida, A., Kobayashi, K., Manya, H., Taniguchi, K., Kano, H., Mizuno, M., Inazu, T., Mitsuhashi, H., Takahashi, S., Takeuchi, M., et al. (2001). Dev. Cell *1*, 717–724.